University of Gondar

College of Natural and Computational Sciences

Department of Chemistry

M.Sc. Thesis

Simultaneous Determination of Paracetamol and Ascorbic Acid Using Activated Glassy Carbon Electrode

By: Yared Shewarega

Advisor: Dereje Yenealem (M.Sc.)

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (Physical)

Gondar, Ethiopia

June 2017

Simultaneous Determination of Paracetamol and Ascorbic Acid Using Activated Glassy Carbon Electrode

Department of Chemistry

College of Natural and Computational Sciences

University of Gondar

By: Yared Shewarega

Advisor: Dereje Yenealem (M.Sc.)

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (Physical)

Gondar, Ethiopia

June, 2017

Thesis Approval Sheet

The thesis titled "Simultaneous Determination of Paracetamol and Ascorbic Acid Using Activated Glassy Carbon Electrode" by Mr. Yared Shewarega is approved for the degree of "Master of Science in Chemistry (physical)".

Examiners

Dr, Muluken A (External examiner)

Dr, Molla T (Internal examiner)

Supervisor/Advisor

Dereje Y (M.Sc.)

Chairman

Dr, Madhusudhan A

Date: 23/06/2017

Place: University of Gondar

Declaration

I, the undersigned, declare that the work reported herein represents my own ideas in my own words and wherever others' ideas or words have been included, I have adequately cited and referenced the original sources. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/fabrication/falsification of any idea/data/fact/source will constitute sufficient ground for disciplinary action by the University and can also evoke penal action from the sources which have not been properly cited or acknowledged.

_____

Signature

Yared S

Name of Student

23/06/2017

Date

Contents Page

Acknowledgments

Above all I express my indebtedness to the "**ALMIGHTY**" for all **His** blessing and kindness.

I would like to express my sincere gratitude and regards to my advisor Mr, Dereje Yenealem (M.Sc.) who supported me by providing proper guidance and advice for the completion of this thesis work.

I would like to extend my special thanks to Dr, Meareg Amare for his generous permission to do the experimental works in Bahir Dar university research laboratory and for taking time to support me with valuable guidance and consistence encouragement throughout the laboratory work.

I would like to show my gratitude to M.Sc. students of physical chemistry streams, Meselu Eskezia, Tekilu Tukye and TilahunYai for their friendship ideas and comments at the very beginning of this thesis work up to finishing of the study.

Finally, I would also like to acknowledge the Ministry of education, for sponsoring me to do my M.Sc with different supports for completing the work.

List of acronyms

AA	Ascorbic acid
AGCE	Activated glassy carbon electrode
ABS	Acetate buffer solution
BDDE	Boron doped diamond electrode
BRBS	Briton Robinson buffer solution
CCE	Carbon ceramic electrode
CPE	Carbon paste electrode
CV	Cyclic voltammetry
DME	Dropping mercury electrode
DPV	Differential pulse voltammetry
EPHARM	Ethiopian pharmacy
GC	Glassy carbon
GCE	Glassy carbon electrode
LOD	Limit of detection
LOQ	Limit of quantification
MWCNTs	Multi-walled carbon nanotub paste electrode
NAPQI	N-acetyl-*p*-quinoneimine
NPV	Normal pulse voltammetry
OSWV	Osteryoung square wave voltammogram
APAP	Acetaminophen/Paracetamol
PBS	Phosphate buffer solutions
PC	Personal computer
Redox	Reduction and oxidation
RSD	Relative standard deviation
SWV	Square wave voltammetry
UA	Uric acid

UV	Ultra violet
WE	Working electrode

List of figures

List of tables

List of Schemes

Abstract

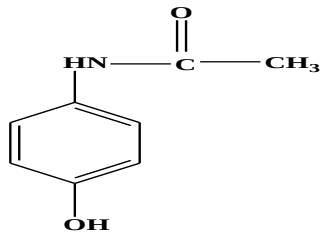
Square wave voltammetric technique (SWV) was employed for the detection of APAP and AA by using activated glassy carbon electrode (AGCE). The AGCE was prepared by activating for 200 s at a potential of 1.750 V, sensitivity; 100 µA/V in 0.1 mol L^{-1} phosphate buffer solution at pH 7.0. It was found that AGCE efficiently electro catalyzed the oxidation of APAP and AA than bare GCE. The cyclic voltammetric study showed that AGCE decreased the overpotential of both paracetamol and ascorbic acid and displayed excellent electrochemical catalytic activities toward APAP and AA compared with bare GCE. The scan rate studies showed that the electrochemical behavior of APAP and AA was controlled by surface adsorption process. In the voltammetry technique, both APAP and AA exhibited sensitive oxidation peaks and gave linear characteristic response. The linear range, quantification and detection limits of APAP were found to be 10 to 100 µmol L^{-1}; 0.517 µmol L^{-1} and 0.155 µmol L^{-1}, respectively. Similarly, the linear range, quantification and detection limits of AA were found to be 0.4 to 0.95 mmol L^{-1}, 6.32 µmol L^{-1} and 1.89 µmol L^{-1}, respectively. The interfering study showed that uric acid interfered in the determination of APAP. The proposed method applied to the determination of APAP and AA in pharmaceutical samples with better results. The proposed method was applied for APAP and AA determination in commercial drug formulation paracetamol + Vitamin C SANDOZ with a recovery of 101.05% and 95%, respectively.

***Key word**: Glassy carbon electrode; Activated glassy carbon electrode; Ascorbic acid; Paracetamol; Adsorption; Cyclic voltammetry; Square wave voltammetry;*

1. INTRODUCTION

1. Background of the study

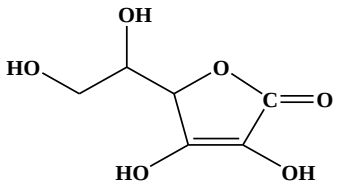
Pharmaceutical drugs serve life in normal therapeutic dose, **[1]** and it is important to develop simple, sensitive, and accurate methods for detecting pharmaceutically electro-active compounds **[2]**. Paracetamol (APAP, **scheme 1**) is a pharmaceutical drug derived from its chemical name, N-acetyl-para-aminophenol **[3]**. Initially, it was found in urine of patients who had taken phenacetin and in 1889 demonstrated that it was a urinary metabolite of acetanilide **[4]**. It is the most popular analgesic and antipyretic drug **[5 - 8]**, and alternatively used as patients for sensitive to other pain killer drugs like; aspirin, cetirizine, tramadol, and codeine **[9]**. In addition, it is used to reduce mild to moderate pains including instances of tension headache, migraine headache, muscular aches, chronic pain, neuralgia, backache, joint pain, general pain and toothache **[10]**. APAP, does not exhibit any extreme health effects, because in normal therapeutic dose easily metabolized and completely eliminated in urine **[11]**. However, the overdoses in some cases can leads to the formation of toxic metabolites, such as; hepatotoxicity, nephrotoxicity, **[11 - 13]** liver disorders, skin rashes and inflammation of the pancreas **[13, 14]**. The daily permitted maximum intake is 4 g **[15]**.



Scheme 1. Chemical structure of paracetamol.

Ascorbic acid (vitamin C, AA, **scheme 2**) discovered by Szent-Gyorgyi (1928), **[16]** and well known by its high antioxidant activity **[17, 18]**. It is a power full water soluble type of organic acid, **[18]** and clinically used for the treatment and prevention of scurvy, common cold, mental illness, cancer, AIDS, and protecting living cells against oxidative injury **[19]**.

Ascorbic acid is non toxic, but in some cases an over doses (2-6g/day) can leads gastrointestinal disturbances, **[20]** such as; abdominal distention, flatulence, diarrhea and transient colic **[16]**.



Scheme 2. Chemical structure of L-ascorbic acid.

Previously analytical methods have been developed and routinely used for the simultaneous determination of APAP and AA. However, some of the methods, such as; chromatography, **[21 - 24]** electrophoresis, **[25]** and spectrophotometry methods, **[26]** were conducted with some disadvantages like; long analysis time, the need for sample preparation, complicated procedures with high cost of investment and exhibit poor performance **[11]**. On the other hand, the electrochemical techniques obtained consideration for the determination of electroactive compounds in pharmaceutical forms and physiological fluids, **[12]**; this is due to their simplicity, selectivity, low cost, short analysis time, reproducibility and sensitivity **[19, 27]**.

Therefore, beside the advantage of the electrochemical methods another very important electrochemical method developed that favors their use, the voltammetric techniques are the most common known for the quantitative determination of pharmaceuticals. They have excellent sensitivity, selectivity, and reproducibility, low detection limit, **[28]** a wide range of temperature, rapid analysis time, the ability to determine kinetic and mechanistic parameters both in identification and quantification of pharmaceuticals **[29]**. In voltammetric determination, cyclic voltammetry conducted for the study of the electrochemical behavior (redox reaction) and the voltammetric methods introduced to enhance the sensitivity and speed in many forms of potential formulation **[30 - 32]**.

Voltammetric techniques are represented by solid or carbon electrode materials and these modified in different ways such as, aluminum electrode modified by thin layer of palladium, **[33]** multi-walled carbon nanotube paste electrode (MWCNTs), **[32]** single-walled carbon nanotub-modified

carbon ceramic electrode, **[34]** gold nanoparticles /multiwalled carbon nanotub /glassy carbon electrode,**[35]** a glassy carbon electrode modified with multi-wall carbon nanotubes dispersed in polyhistidine, **[36]** thionine immobilized multi-walled carbon nanotube modified carbon paste electrode, **[37]** unmodified boron doped diamond electrode, **[38]** and n-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide-modified carbon nanotubes paste electrode, **[39]** have been reported for the simultaneous determination of APAP and AA. Even though, better results were recorded on determination of the two analytes (APAP and AA), some factors, such as; time, labor and materials required to modify the electrodes makes the technique cost ineffective. In this study, the potentially activated glassy carbon electrode was used for the simultaneous determination of APAP and AA, to the best the work; no results have been reported about the possibility to simultaneous determination of APAP and AA using potentially activated GCE. It was found that APAP and AA appeared two well defined oxidation peaks at the activated electrode. A new method for the simultaneous determination of APAP and AA concentrations were developed in pharmaceutical formulation form.

2. Statement of problem

Drug analysis is an important branch of chemistry and plays an important role in drug quality control. The main aim of the pharmaceutical drugs is to serve the human to make them free from potential illness or prevention. However, the problems associated with chemical composition, quality, quantity and toxicity in daily dosage form not solved yet. Previously, analytical methods have been developed using different types of modified electrodes to reduce the over potential for the catalytic electro-oxidation of active compounds. However, the electrode behavior degrades with time due to the adsorption of impurities from the solution and the solid electrode becomes unsuitable for quantitative measurements in drug quality control. To overcome the problems, the study was conducted by introducing a simple electrochemically activated glassy carbon electrode without employing any expensive modifier.

3. Significance of the study

The development of a new method to determine pharmaceutical dosage forms is important. The study provided new information to quantify and qualify pharmaceutical drug on health care. The result of the study gives information about how the selected technique is less toxic, cheap, eco-

friendly but equally sensitive electrochemical method on quantitative determination of APAP and AA for regular quality control purpose in laboratories. Additionally, the result of the study provided adequate information those who are interesting to work on similar work with different pharmaceutical drug formulations.

1. Objective of the study

1. General objective

The main objective of the study is to determine paracetamol and ascorbic acid using activated glassy carbon electrode.

2. Specific objectives

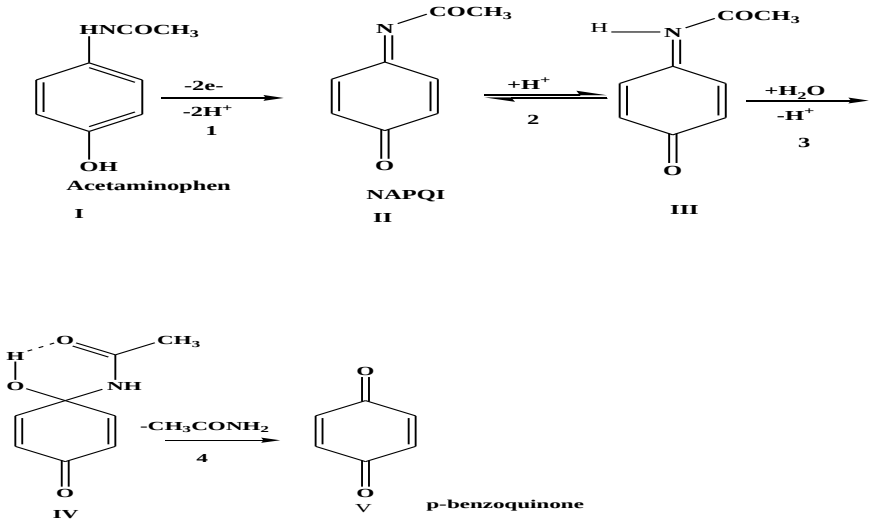
1. To investigate the significance of AGCE of APAP and AA than bare glassy electrode
2. Describe the electrochemistry of APAP and AA at phosphate buffer solution at different pH values and scan rates (*v*)
3. Distinguish the type of reaction is whether diffusion or surface adsorption controlled process
1. Compare the results method with other methods
2. To study the influence of interferences in the determination of APAP and AA

1. LITERATURE REVIEW

1. Mechanism of electrochemical oxidation of APAP and AA

1. Mechanism of electrochemical oxidation of APAP

The electrochemical oxidation mechanism of paracetamol (**Scheme 3**) proceeds by a two electron, two proton processes, and the result is *N*-acetyl-*p*-quinoneimine (NAPQI) and the final product is *p*-benzoquinone **[4]**.



Scheme 4. Electrochemical process for the oxidation of paracetamol.

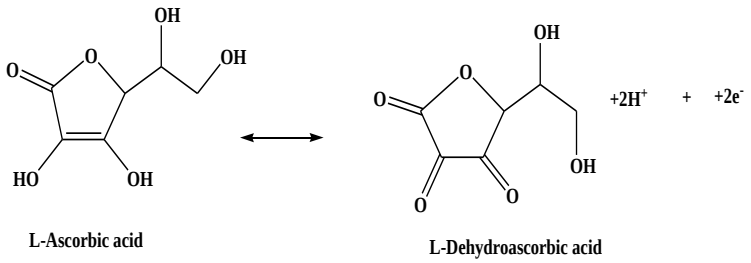
Paracetamol is oxidized in a quick pH to give NAPQI and at a pH greater than or equal to 6.0 NAPQI (II) exists in its stable state. Under more acidic conditions, NAPQI is readily protonated to give species (III), which is a less stable, but electrochemically active species. Species (III) then rapidly yields a hydrated species (IV), which is electrochemically inactive in the examined potentials. Finally, under increasingly more acidic conditions the hydrated species (IV) converts

to benzoquinone (V), and only under extremely acidic conditions will the reduction of benzoquinone observed with cyclic voltammetry **[40]**.

2. Mechanism of electrochemical oxidation of AA

Ascorbic acid is a water soluble compound consisting of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L- dehydroascorbic acid. It is an excellent source of electrons and donates electrons to free radicals such as hydroxyl and super oxide radicals and quenches their activity **[16]**.

There have been difficulties in quantifying ascorbic acid due to its instability in aqueous solution or due to its oxidation to dehydroascorbic acid, which is a reversible reaction **[41]**. The oxidation of AA has been widely agreed to follow the electrochemical method mechanism and the process involves the loss of two electrons at pH (1 - 4), one proton at pH > 5 **(Scheme 5)**, **[17]** and the final species is electro inactive, which explains the absence of peak at the reverse scan **[42]**.



Scheme 6. Electrochemical process for the oxidation of ascorbic acid.

1. Physicochemical properties of APAP and AA

1. Physicochemical properties of APAP

Research on medicinal products stored in different temperatures and humidity has shown that physical properties of paracetamol ($C_8H_9NO_2$) in the form of powder and solution remain unaffected **[17]**. The appearance of APAP is white, odorless crystalline compound with a bitter taste, **[39]** and it is soluble in organic solvents such as; methanol and ethanol but slightly soluble

in water and ether. Its melting point is 169 - 170 ^{O}C and the stability decreases at high or low pH value **[43]**. APAP is a weak acid (pKa = 9.5) in aqueous media, and the most stable at saturated aqueous solutions **[14]**.

2. Physicochemical properties of AA

The physicochemical properties of ascorbic acid ($C_6H_8O_6$) are related to its structure (**Scheme 2**). The stability of AA decreases with increased temperature, sun exposure and pH. The appearance of $C_6H_8O_6$ is white, odorless, crystalline solid with sharp acidic state **[44]**. It contains several structural elements that contribute to their chemical behavior: the structure of the lactones and two enolic hydroxyl groups, a primary and secondary alcohol group. Enediol structure motivates their antioxidant properties, as can be oxidized easily enediols to diketones. AA is rapidly interconvert in to unstable diketone tautomers by proton transfer, though it is most stable in the enol form. The proton of the enol is lost, and again acquired by the electrons from the double bond to produce a diketone **[45]**.

2. Activated glassy carbon electrode (AGCE)

The aim of the electrode is to enhance both selectivity and sensitivity in the electrochemical determination of pharmaceutically and biologically electro-active compounds **[46]**. There are number of carbon based electrodes obtained in variety forms used for the electro-analytical studies of oxidizable compounds **[39, 47]**. The most commonly used carbon based electrode in the analytical laboratory is glassy carbon (GC). It is made by pyrolyzing a carbon polymer, under carefully controlled conditions, to a high temperature like 2000 ^{0}C **[48]**.

Glassy carbon electrode (GCE) has been used as electrode material due to its excellent electrical and mechanical properties, wide potential range, extreme chemical inertness, being highly resistant to acid attack, impermeable to gases and has relatively reproducible performance **[49]**. Many strategies have been developed using different types of modified electrodes to reduce the over potential for the catalytic electro-oxidation of active compounds **[50]**. However, the electrode behavior degrades with time due to the adsorption of impurities from the solution or chemical changes to the electrode surface and the solid electrode becomes unsuitable for quantitative measurements.

To reduce the problem different methods have been applied such as; laser treatment and vacuum heat treatment **[51]**. Even though, the electrochemical treatment of glassy carbon electrodes obtained considerations, **[52]** due to cost effectiveness, lower background current, noise levels and low detection limit than other carbon materials [**53]**.

3. Theoretical background of electrochemical techniques

Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as large linear dynamic range, with relatively low cost instrumentation **[54]**. The application of electrochemical techniques in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. The renewed interest in electrochemical techniques can be attributed in part to more sophisticated instrumentation and to increase the understanding of the technique themselves **[1]**. The techniques have shown remarkable advantages in the analysis of drugs in pharmaceutical preparations, and can easily solve many problems of pharmaceutical interest. The most useful electro-analytical techniques are based on the concept of continuously changing the applied potentials to the electrode solution interface and the resulting measured current **[55]**.

1. Voltammetric techniques

Voltammetry is an electro-analytical technique conducted by measuring the current flowing through an electrode dipped in solution containing electro-active compounds while a potential is imposed upon it. The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (I) flowing through the electrochemical cell. The electrochemical cell, where the voltammetric experiment is carried out, consists of a working (indicator) electrode, a reference electrode, and a counter (auxiliary) electrode **[56]**.

The voltammetric techniques have been applied for the determination of pharmaceutically electro-active compounds in dosage forms (tablets, capsules, injections and suspension) and biological samples (real and spiked urine samples, blood and serum). The short analysis time in these methods makes it very attractive for routine determination of the analytes in different samples.

In addition, the analytical advantages of the various voltammetric techniques include excellent sensitivity, a large number of useful solvents and electrolytes, a wide range of temperatures, rapid

analysis times, simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents recorded **[29]**.

1. Cyclic voltammetry (CV)

Cyclic voltammetry is the most common known voltametric technique and popularly used to study the electro-chemical properties of an analyte in a solution, and rarely used for quantitative determination of pharmaceutically and physiologically electro-active compounds. CV is a rapid voltage scan technique in which the direction of voltage scan is reversed, and the scan rate in the forward and reverse direction is the same. The measured parameters in cyclic voltammetry are anodic and cathodic peak potential (E_{pa} and E_{pc}), anodic and cathodic peak current (I_{pa} and I_{pc}) and the half peak potentials ($E_{p/2}$) at which the cathodic and anodic currents reach half of their peak value (**Fig.1**) **[54]**.

In cyclic voltammetry experiment the potential of the working electrode is ramped at a scan rate (v) and the resultant trace of current against potential is plotted in what is called a voltammogram **[32]**. In addition, cyclic voltametry techniques are widely used a powerful technique for the study of reversible, irreversible and quasi-reversible redox reaction. In CV the potential is ramped from an initial potential (E_i) and at the end of its linear sweep. The direction of the potential scan is reversed, usually stopping at the initial potential. The potential at which the change in direction occurs is also known as the switch potential (E_λ) **[31]**.

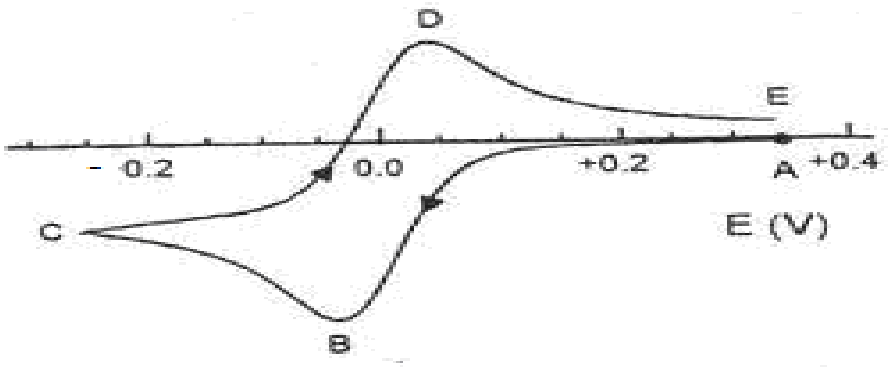


Figure 1. A typical cyclic voltammogram exhibited by a species which undergoes a reversible reduction at $E^{o\prime} = 0.00$ V.

Where, A represents the initial potential, B the cathodic peak, C the switch potential, D the anodic peak and E final potential.

If the potential is scanned from a positive to a negative value, then reduction would occur during the forward part of the CV scan and oxidation during the reverse CV scan. For a reversible reaction the CV usually has two peaks, one for each of oxidation and reduction taking place. The position of both the cathodic and anodic peaks gives thermodynamic information of the redox couple used. The anodic and cathodic peak potentials used to calculate the formal electrode potential, $E^{o\prime}$ as follows;

……..……..1

Where, $E^{o\prime}$ = the formal electrode potential /standard electrode potential, E_{pc} = cathodic peak potential, E_{pa} = anodic peak potential.

The Randles-Sevčik equation is obeyed if a plot of peak current (I_p) against analyte concentration (C_{analyte}) yields a straight line.

…… 2

At 25 ^{0}C and constant solvent, swamping electrolyte the equation changed in to,

……. 3

Where, n = number of electrons transferred, F = Faraday constant (96485.339 C/mol), A = the electrode surface area in (m^2), v = scan rate in (Volt/s), R = gas constant (8.314 J/K), T = Temperature (K) and C = concentration (mol/cm^3) and D = diffusion coefficient (m^2/s) **[30, 31]**.

2. Normal pulse voltammetry (NPV)

NPV technique consists of a series of potential pulses of increasing amplitude. The measurement of peak current produced in usually carried out at near the end of each pulse, and the current measurement is made near the end of each pulse, which allows time for the charging current to decay. The potential is pulsed from an initial potential E_i and duration of the pulse, t, is usually 1 to 100 m sec and the interval between pulses typically 0.1 to 5 sec. The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis (**Fig. 2**). In NPV technique at certain period the resulting current is zero, this may be explained as between the pulses i.e., the electrode is kept at a constant potential at which there is no chemical reaction occurs in the cell **[29, 54]**.

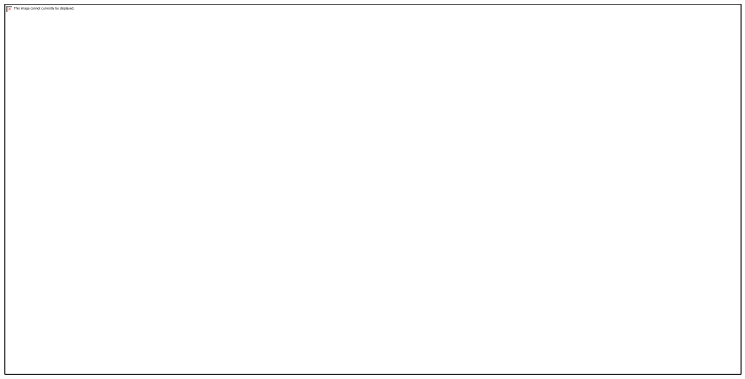


Figure 3. Typical voltammogram in Normal pulse voltammetry.

3. Differential pulse voltammetry (DPV)

The DPV technique is better than the NPV, because the peaks are sharper and better defined at lower concentration with a lower background current and improved resolution **[57]**. In addition, DPV is effective and rapid electro-analytical technique with well established advantage, including low detection limits **[58]**.

It is comparable to NPV in that the potential is scanned with a series of pulses however, each potential pulse is fixed of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and the second (2) at the end of the pulse. As shown from **Fig.3** the difference between current measurements at these points for each pulse is determined and plotted against the base potential **[29]**.

Its principal advantages over normal pulse voltammetry are twofold: (i) many analytes can be sampled with a single voltammogram since the analytical peaks for each analyte are quite well resolved, and (ii) by working with a differential current, and hence obtaining a voltammetric peak, the analytical sensitivity can be improved and the sensitivity is clearly superior to NPV **[59]**. DPV is particularly useful to determine accurately the formal electrode potentials of partially overlapping consecutive electron transfers and it affords symmetric peaks which start from zero current and finish at zero current values **[30]**.

1. **(b)**

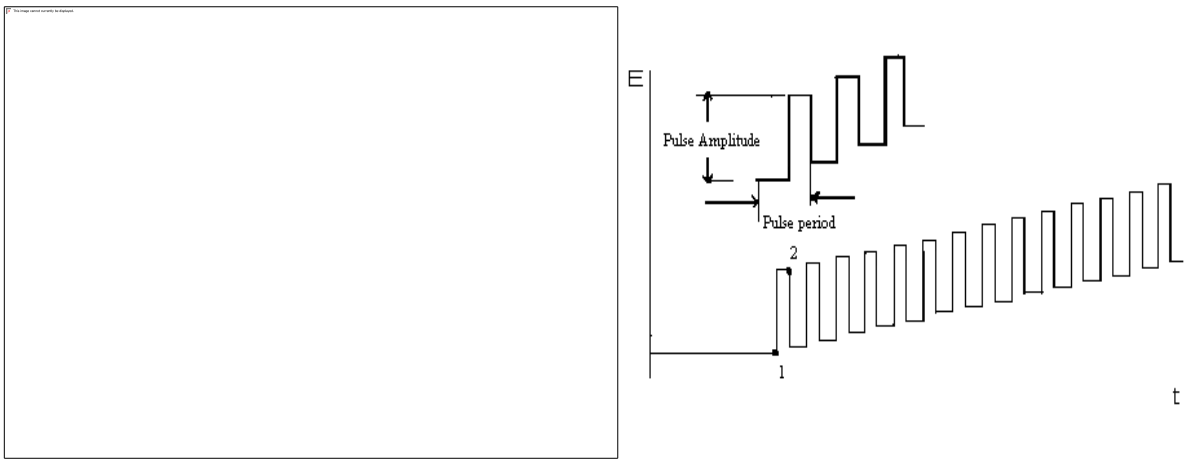


Figure 4. (a) Typical voltammogram of DPV and (b) Excitation waveform of DPV.

1. Square wave voltammetry (SWV)

Square wave voltammetry is the most effective and rapid voltammetric technique with well established advantages, including good discrimination against background currents and low detection limits **[60]**. Its highest sensitivity and speed is due to high scan rate in all cases where the reacting species is accumulated by adsorption on the electrode surface. Comparison of square wave (SW) and DPV for reversible and irreversible cases indicated that the SW currents are 4 and 3.3 times higher, respectively, than the analogous differential pulse response **[54]**.

The excitation signal in SWV consists of a symmetrical square wave pulse of amplitude (E_{sw}) superimposed on a staircase waveform of step height (ΔE), where the forward pulse of the square wave coincides with the staircase step. The net current (I_{net}), is obtained by taking the difference between the forward and reverse currents. The peak height is directly proportional to the concentration of the electro-active species and direct detection limits as low as 10^{-8} mol L^{-1} **[29]**. The voltamogram (**Fig.4a**) is peak shaped, but it consists of a differential curve between the current recorded in the forward half cycle and the current recorded in the reverse half cycle **[30]**.

(a) (**b)**

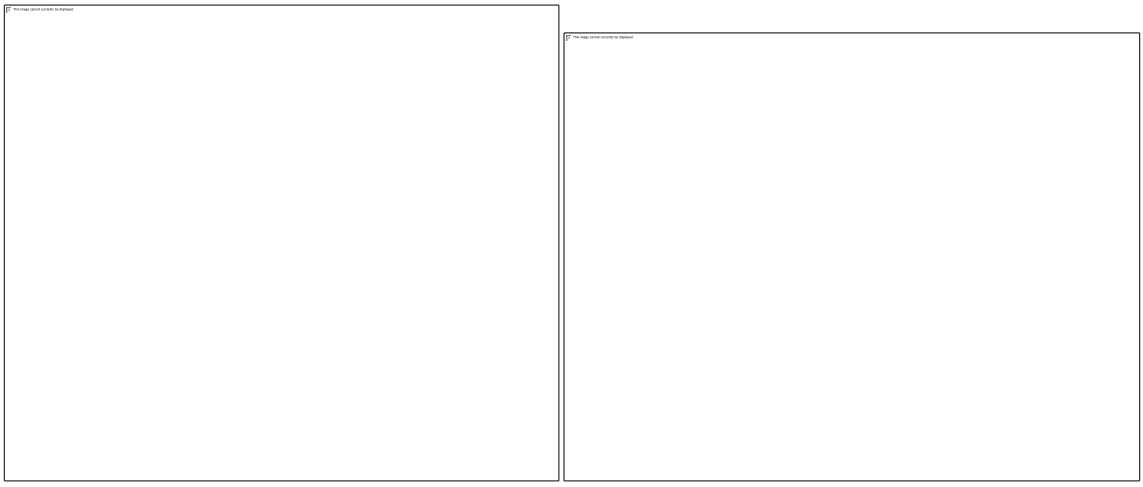


Figure 5. (a) Typical Osteryoung square wave voltammogram (OSWV) and (b) applied potential waveform in SWV.

1. EXPERIMENTAL SECTION

1. Instrumentation

Voltammetric experiments were carried out using voltammetric analyzer CHI760E (Bioanalytical systems (BAS, USA) equipped with voltammetric interface and driven by software package in conjunction with a three electrode system and a dell desk top computer was employed for data storage and processing. A three electrode cell system composed of silver/silver chloride (Ag/AgCl) as the reference electrode, a platinum wire as the counter/auxiliary electrode, and the bare and potentially activated glassy carbon electrodes as the working electrodes each 3 mm diameter were used. The pH meter (JENWAY model 3510) and digital balance were used.

2. Chemicals and reagents

Pure paracetamol (Addis pharmaceutical factory, Ethiopia), pure ascorbic acid **(**Tarapu MIDC, India), paracetamol + vitamin C SANDZ (Europe), anhydrous di-potassium hydrogen orthophosphate (BDH, England), potassium dihydrogen phosphate (Sigma Aldrich, Switzerland), hydrochloric acid (Riedel deHaen, Germany), uric acid (LABORT, India), and sodium hydroxide (BDH, England) without any further purification were used. The stock solutions of paracetamol and ascorbic acid were prepared and kept in a refrigerator until used. An aqueous solutions of paracetamol and ascorbic acid were prepared daily of the working days by simple dilution of the stock solution with pH 7.0 phosphate buffer solution (PBS). PBS (0.1 mol L^{-1} KH_2PO_4 - 0.1 mol L^{-1} K_2HPO_4) was prepared by using distilled water, and the pH of the PBS was adjusted by adding drop by drop of concentrated hydrochloric acid and sodium hydroxide.

3. Analytical procedures

1. Preparation of the activated glassy carbon electrode (AGCE)

Before activation, the surface of glassy carbon electrode (3.0 mm diameter) was polished to a mirror with 0.5 μm alumina powder with in a polishing cloth and then cleaned thoroughly with distilled water. The cleanness of the electrode was checked by a 0.5 mol L^{-1} sulfuric acid by

running in cyclic voltammetry with a potential window between -800 mV and 800 mV at the scan rate of 100 mV/s, sensitivity; 100 µA/V. Then, the glassy carbon electrode was activated for 200 s in a time base technique at a potential of 1750 mV, sensitivity; 100 µA/V in 0.1 mol L^{-1} PBS at pH 7.0. Then cyclic voltammogram of the activated electrode was run between a potential from 0.0 to 700 mV for six cycles. The activated electrode was run in cyclic voltammetry until the voltammograms was stabilized.

2. Preparation of standard solutions

For cyclic voltammetric studies, 1 mmol L^{-1} APAP and 1 mmol L^{-1} AA stock solutions were prepared by dissolving 0.0755 g of APAP and 0.0882 g of AA in 500 ml of 0.1 mol L^{-1} pH 7.0 phosphate buffer solutions. From the stock solutions, 0.1 mmol L^{-1} solution of APAP, 0.5 mmol L^{-1} solution of AA and a mixture (0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA) were used for the voltammetric investigation of APAP, AA and the mixture both at the bare and the activated glassy carbon electrodes. The working solutions of different concentrations of APAP (10, 20, 40, 60, 80, 100, 140 and 180 μmol L^{-1}) and AA (0.1, 0.15, 0.2, 0.25, 0.4, 0.55, 0.75 and 0.95 mmol L^{-1}) in pH 7.0 PBS were prepared from stock solutions of 1 mmol L^{-1} APAP and 1 mmol L^{-1} AA through serial dilution, respectively.

3. Sample preparation from paracetamol + vitamin C SANDOZ

The simultaneous determination was done by a pharmaceutical drug containing both APAP and AA pharmaceutically called paracetamol + vitamin C SANDOZ (500 mg APAP / 300 mg AA). An adequate amount of the powder (paracetamol + vitamin C), which is equivalent to the standard powder was accurately weighed and added in to 100 m L volumetric flask, and diluted with 0.1 mol L^{-1} pH 7.0 phosphate buffer solution.

The flask was thoroughly shaken until the sample dissolved and the mixture was filled to the appropriate volume with the buffer solution. Standard solutions in paracetamol + vitamin C SANDOZ were prepared by spiking of the drug into an aqueous stock solution of standard paracetamol and ascorbic acid samples.

4. Electrochemical experiments

Voltammetric determination of paracetamol and ascorbic acid was carried out in a voltammetric cell consisting of a three electrode system, including bare GCE and AGCE as the working electrode, a platinum wire electrode as a counter/ auxilary electrode, and Ag/AgCl as a reference electrode with 0.1 mol L^{-1} PBS (pH 7.0) as a supporting electrolyte solution. The electrochemical behavior of pure paracetamol and pure ascorbic acid were investigated by using cyclic voltammetry. The SWV determination was carried out by scanning the potential in the range from -200 mV to +800 mV with SWV parameters; amplitude = 25 mV, frequency = 15Hz, quiet time = 2 s and sensitivity = 10 µA/V. The detection limits of APAP and AA were calculated as three times of the standard deviation of the blank phosphate buffer solution pH 7.0 of APAP and AA divided by the slope of the calibration curve of APAP and AA, respectively. Similarly, the limit of quantifications were calculated as ten times of the standard deviation of phosphate buffer solution pH 7.0 of APAP and AA divided by the slope of the calibration curve of APAP and AA, respectively. Distilled water was used for all analyses. The electrochemical experiments were carried out at room temperature (25 ^{0}C).

2. RESULTS AND DISCUSSION

1. Electrochemical behavior of APAP and AA

Fig. 5 shows cyclic voltammograms of the bare GCE **(a)** and the activated glassy carbon electrode (**b**) in the presence of 0.5 mmol L^{-1} AA in a 0.1 mol L^{-1} pH 7.0 PBS that was recorded at a scan

rate of 100 mV s^{-1}. As can be seen (curve **a**) the cyclic voltammogram of the bare glassy carbon electrode in the presence of 0.5 mmol L^{-1} AA no well resolved oxidative and reductive peaks were observed in the potential range from -0.2 to 0.8 V. The broad oxidative peak current is observed without any reductive peak indicating that the electrochemical reaction of ascorbic acid at bare GCE is totally irreversible, and no reproducible electrode response is obtained due to fouling of the electrode surface by the adsorption of the oxidized product of AA.

On the other hand, the anodic peak potential (E_{pa}) for the oxidation of AA on the surface of the activated glassy carbon electrode (AGCE) **(b)** occurs at 20 mV and the anodic peak current is enhanced in comparison with the anodic peak current on untreated GCE. This shows that the oxidation of ascorbic acid on AGCE irreversible. Moreover, the oxidation peak shift to less positive potential (20 mV), shows that much lower over-potential (when compared to the bare GCE) is required for the electron transfer. Besides, the shift in the AA oxidation potential with a concomitant increase in the peak height reflects a faster electron transfer reaction of ascorbic acid. Hence, the faster electron transfer leads to a sharper and better defined peak, as the control of the process passes from the electron transfer to the mass transport **[34]**.

Figure 6. Cyclic voltammograms of 0.5 mmol L^{-1} AA at the bare GCE (a) and the AGCE (b) respectively in 0.1 mol L^{-1} PBS pH 7.0 scan rate; 100 mV s^{-1}.

Fig. 6 shows the cyclic voltammograms of APAP at the surface of the bare GCE (**a**) and AGCE (**b**). As can be seen, **Fig. 6a** APAP shows an oxidative peak potential at E_{pa} = 550 mV and a small cathodic peak potential at E_{pc} = -83 mV, with peak separation between the anodic and cathodic peak potential (ΔE) is 633 mV. The cyclic voltammogram of APAP at the surface of AGCE (**b)** shows a pair of well defined redox peaks with anodic and cathodic peak potentials at 360 and 340 mV, respectively and the change in potential is 20 mV. On the other hand, the anodic peak potential of APAP shifts from 550 mV at the bare GCE to 360 mV at the AGCE, and the anodic peak current (I_{pa}) is enhanced by approximately 3- fold compared at bare GCE. The decrease of oxidation over potential (190 mV) and the peak current enhancement clearly indicated that activation had great catalytic performance for electrochemical reaction of APAP. This is due to rapid electron transfer rate on activated surface, indicating activation changes the oxidation of APAP from irreversible (slow electron transfer) to a reversible reaction (fast electron transfer).

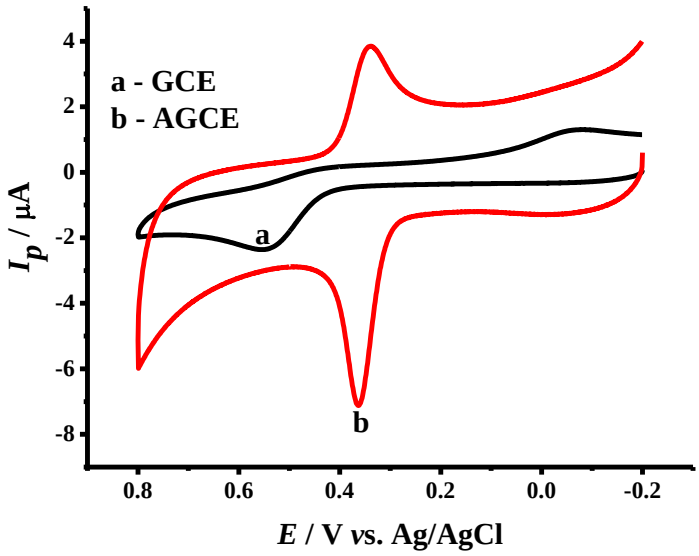


Figure 7. Cyclic voltammograms recorded 0.1 mmol L^{-1} APAP at the bare GCE (a) and at the AGCE (b) respectively in 0.1 mol L^{-1} PBS pH 7.0, scan rate; 100 mVs^{-1}.

In general, as shown, **Fig. 5 and 6** the decrease of oxidation over potential accompanied by a significant increase in the oxidation peak current of AA and smaller peak separation for APAP, strongly indicates excellent catalytic ability of the selected method on the oxidation of APAP and AA, respectively.

Table 1. Peak currents and potentials of APAP and AA at bare GCE and AGCE taken from Fig. 5 and 6.

Analytes	Bare GCE					AGCE				
	Peak current (μA)		Peak potential (mV)		Δ*E* (mV)	Peak current (μA)		Peak potential (mV)		Δ*E* (mV)
AA	-				-	I_{pa}	8.5	E_{pa}	20	
APAP	I_{pa}	2.35	E_{pa}	550	633	I_{pa}	7.13	E_{pa}	360	
	I_{pc}	1.32	E_{pc}	-83		I_{pc}	3.92	E_{pc}	340	20

Fig. 7 shows the cyclic voltammograms of a mixture solution of APAP and AA at the bare GCE **(1)** and at the AGCE **(2)**. In therapeutic use, APAP is often found in associated with AA and other pharmacologically and biologically active compounds. The co-presence of APAP and AA leads to diminished toxicity and intensified positive effects of APAP **[61]**. However, as shown from **Fig. 7(curve 1)** the simultaneous electrochemical detection of APAP and AA on bare glassy carbon electrode (GCE) was difficult due to the oxidation of ascorbic acid that takes place at a potential close to that of paracetamol, resulting in an overlapped voltammetric response and low currents due to the absence of electro-catalytic behavior of the bare electrode toward APAP and AA. As can be seen, **Fig. 7 (curve 1)** the overlapped oxidation peak (500 mV) and the presence of low current response at the bare glassy carbon electrode, indicating slow electron transfer (totally irreversible) kinetics on simultaneous determination of APAP and AA. On the other hand, at the surface of the AGCE, the oxidative voltammetric peaks of APAP and AA appear at 360 and 20 mV in PBS (pH 7.0), respectively **(curve 2)**. The larger separation of the peak potentials, the peak current enhancement and the drop of oxidation over potentials for both (APAP and AA) in

comparison with bare GCE representing an improvement in the reversibility of APAP electro-oxidation and suggesting that it was possible to determine both APAP and AA simultaneously at AGCE.

Table 2. Peak current and peak potential of APAP and AA at bare GCE and AGCE.

	APAP					AA	
Electrode	E_{pa} (mV)	E_{pc} (mV)	ΔE (mV)	I_{pa} (µA)	I_{pc} (µA)	E_{pa} (mV)	I_{pa} (mV)
AGCE	360	340	20	14.1	4.43	20	10.66

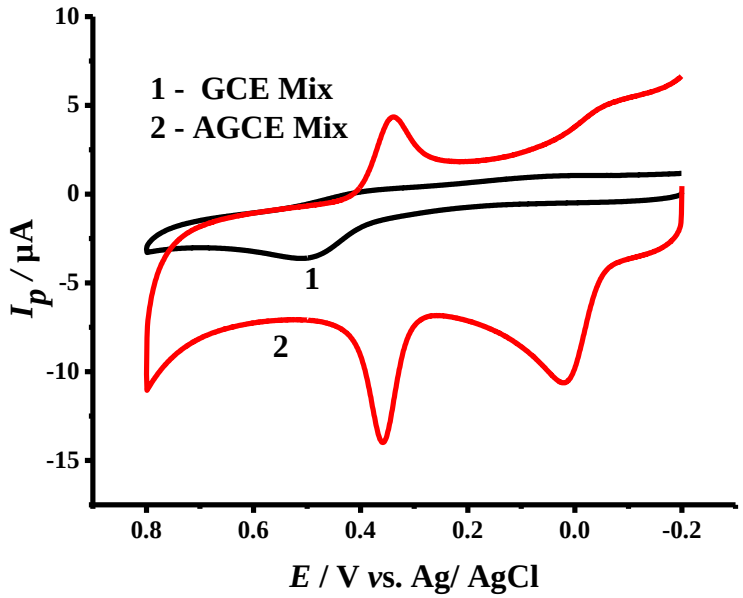


Figure 8. Cyclic voltammograms recorded at a mixture solution of APAP (0.1 mmol L^{-1}) and AA (0.5 mmol L^{-1}) at the bare GCE (1) and at the AGCE (2) respectively in 0.1 mol L^{-1} PBS pH 7.0, scan rate; 100 mV s^{-1}.

2. Effects of pH and scan rate

1. Effect of pH

The pH value of the solution has a significant influence on the peak current and peak potential because of the involvement of protons in the overall electrode reaction. The effects of pH of the

buffer solution on the voltammetric behavior of the mixture containing 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA was investigated in a wide pH range (2.0 – 10.0). As shown, **Fig. 8** the peak potentials shifted towards negative values as the pH increased due to proton involved in electrochemical reaction of APAP and AA **[62]**. In addition, the oxidation peak current of APAP and AA increased as the pH of the solution changes from 2.0 to 7.0 and decreased, then after. As a result, pH 7.0 was selected as optimum value for subsequent studies of APAP and AA.

The deviation at higher pH, indicating that deprotonation or no longer an equal number of protons and electrons process and, suggesting that oxidation reactions of APAP and AA are kinetically less favorable at higher pH. Furthermore, the decreasing of the peak currents at high solution pH could be due to the electrostatic repulsion between APAP (AA) and the activated surface **[63]**.

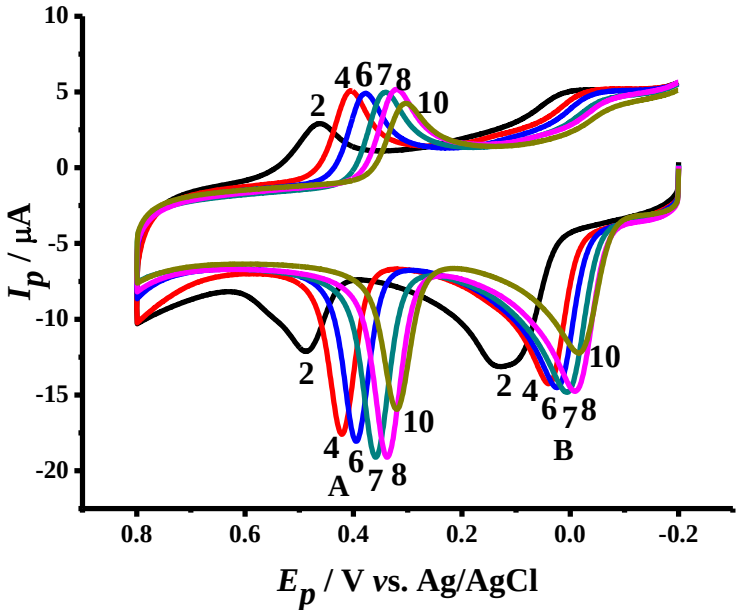


Figure 9. Cyclic voltammograms of pH values on the peak current (I_p) and peak potential (E_p) of 0.1 mmol L^{-1} APAP (A) and 0.5 mmol L^{-1} AA (B) at AGCE, Sensitivity; 100 µA/V.

As shown, **Fig. 9(A)** the oxidative peak potentials of 0.1 mmol L^{-1} APAP **(upper line)** and 0.5 mmol L^{-1} AA **(lower line)** shifted negatively in a linear range of pH (4.0 to 8.0) with a linear regression equation of E_{pa} (V) = -0.02286pH + 0.52286, (R^2 = 0.99068), and E_{pa} (V) = -0.01150pH + 0.08714, (R^2 = 0.99610), respectively and suggesting that equal number of protons and electrons

were involved in the redox reactions of APAP and AA. In addition, **Fig. 9(B)** shows the oxidation peak current of APAP and AA increased up to optimum pH 7.0 and decreased. Based on these observations and in order to obtain high selectivity and sensitivity phosphate buffer solution at pH 7.0 was chosen as optimum supporting electrolyte and used in further experiments.

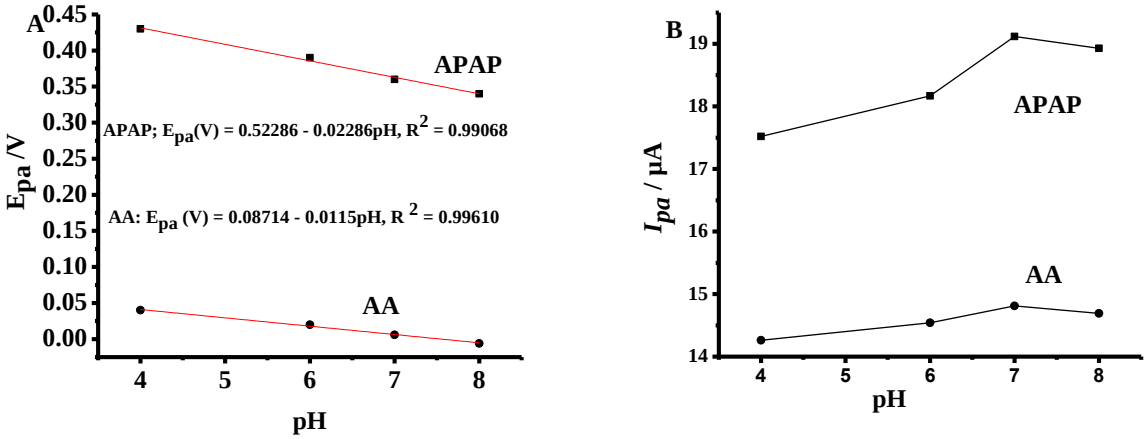


Figure 10. (A) Effect of pH on the peak potential and (B) peak current for the oxidation of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA in 0.1 mol L^{-1}PBS using AGCE at scan rate; 100 mV s^{-1}.

2. Effect of scan rate

The effect of scan rate on the redox peak current of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA at the surface of the activated GCE in 0.1 mol L^{-1} PBS pH 7.0 was investigated. As shown **Fig. 10** the shifting of the peak potentials to more positive values and the proportionality of the peak currents as the scan rate increased, indicating that electron transfer (the catalytic reaction) was controlled by adsorption process. **Fig. 11A** and **B** the peak currents (I_p) of paracetamol and ascorbic acid increased in a scan rate changes from 10 - 300 mV/s and the square root of the scan rate changes from √10 - √300 mV s^{-1} respectively. However, the linear relationship between the peak current (I_p) *verses* scan rate 10 to 300 mV s^{-1} (**Fig. 11)** with a correlation coefficient of 0.99406 for APAP and 0.99619 for AA, indicating that the catalytic reactions were controlled by adsorption process. In addition, the shifting of the peak potentials to the positive values for both APAP and AA with increasing scan rate indicating quasi-reversible and irreversible nature of the activated electrode respectively.

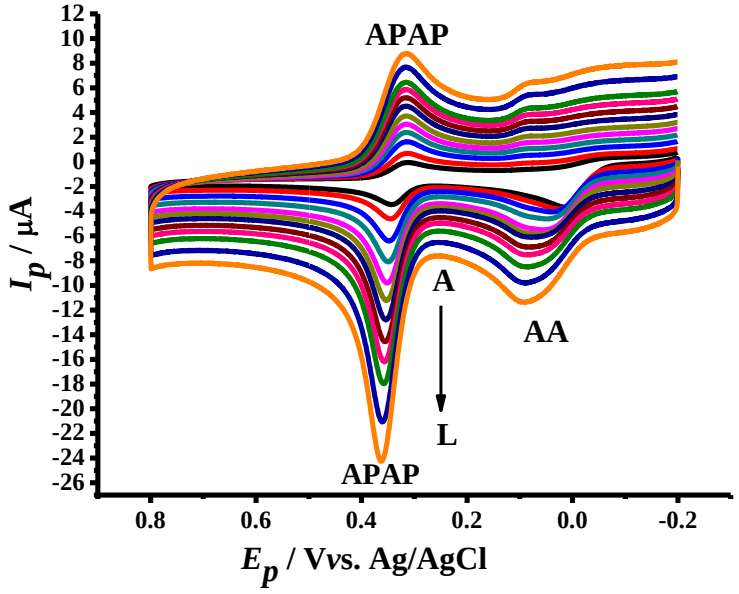


Figure 11. Cyclic voltammograms of scan rate values on the peak current and peak potential of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA with different scan rate (10 - 300) mV s^{-1} using AGCE, sensitivity; 100 µA/V.

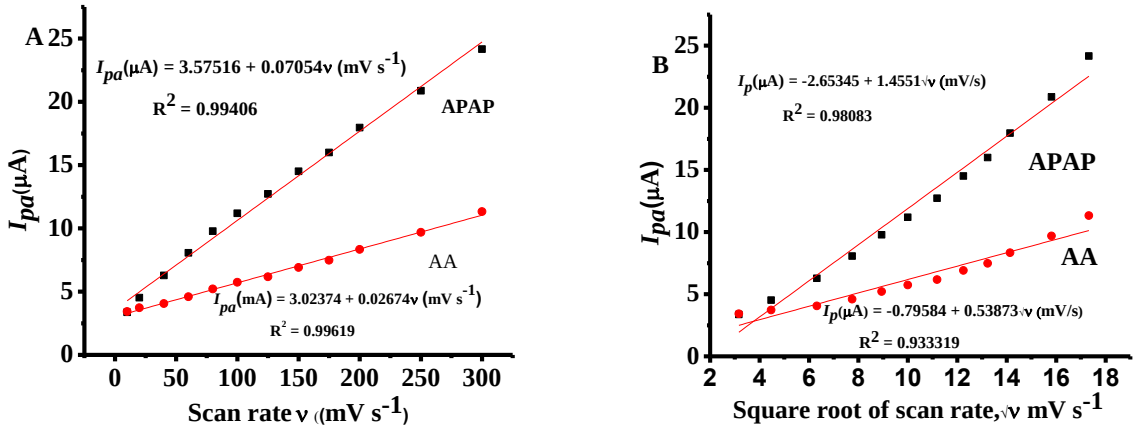


Figure 12. Peak current of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1}AA on the scan rate in the range 10 mV s^{-1} - 300 mV s^{-1} (A), and √10 mV s^{-1} - √300 mV s^{-1} (B), in 0.1 mol L^{-1} PBS pH 7.0 at AGCE.

3. Square wave voltammetric behavior of APAP and AA

To further enhance sensitivity and lower detection limit square wave voltammetry (SWV) was used to detect APAP and AA using AGCE. It has a low charging contribution to the background current, and used to estimate the lower limit of detection. **Fig. 12** shows the simultaneous square wave voltamogram response of APAP (0.1 mmol L^{-1}) and AA (0.5 mmol L^{-1}) on the surface of the AGCE. As can be seen from **Fig. 12**, two sharp and well resolved anodic peaks at 330 mV and -20 mV appears, representing APAP and AA, respectively. The resultant separation in two peak potentials is sufficient (350 mV) to achieve accurate simultaneous determination of APAP and AA in mixtures **[34]**.

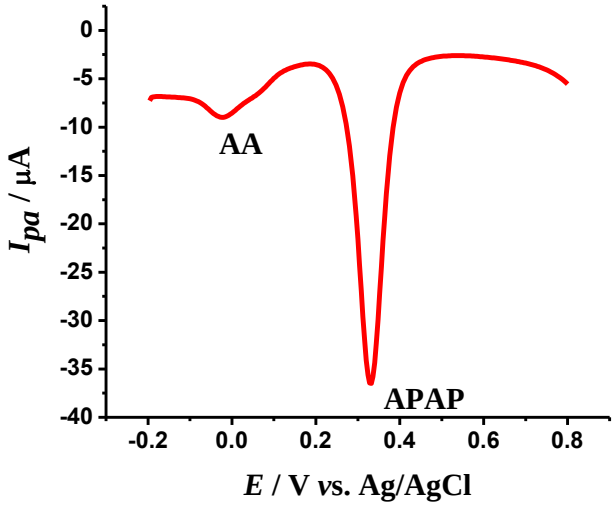


Figure 13. Square wave voltammogram at AGCE in 0.1 mol L^{-1} PBS pH 7.0 containing of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA; SWV conditions were; amplitude = 25 mV, frequency = 15Hz, quiet time = 2 s, sensitivity = 10 µA/V.

Similarly, the electrooxidation processes of APAP and AA in mixtures were investigated when the concentration of one species changed and the other was kept constant. The results show that the peak currents increased linearly with increasing the concentration of one species by keeping the other constant. **Fig. 13A** shows the square wave voltammograms recorded for different concentration of APAP (10 – 180 µmol L^{-1}) at a fixed concentration of AA (0.55 mmol L^{-1}). However; the addition of APAP does not affect the determination of ascorbic acid at the activated surface.

Fig. 13B shows a regression equation in the concentration range of APAP (10 - 180 µmol L^{-1}): I_{pa} (µA) = -5.97761 + 0.51121C (µmol L^{-1}), R^2 = 0.97514 and a linear characteristic was observed in the concentration range of APAP from 10 to 100 µmol L^{-1}. This shows that activated surface effectively utilize the determination of paracetamol in the presence of ascorbic acid.

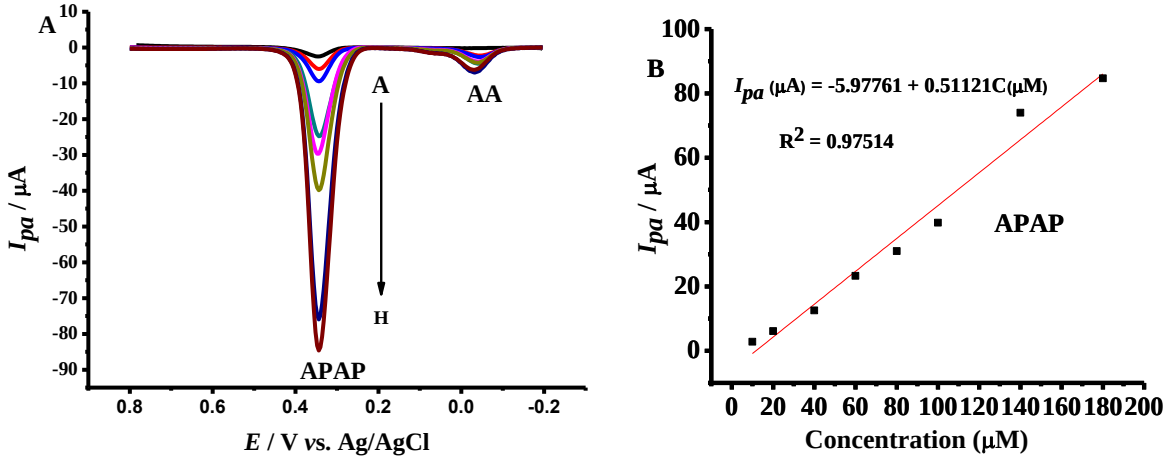


Figure 14. (A) Square wave volatammograms of APAP at AGCE in the presence of (0.55 mmol L^{-1}) AA in 0.1 mol L^{-1} PBS pH 7.0, APAP concentrations (from 1 to 8): 10, 20, 40, 60, 80, 100, 140, and 180 µmol L^{-1}. (B) Calibration plot of anodic peak current I_{pa} (µA) *versus* concentration of APAP (µmol L^{-1}), other conditions as **Fig. 12.**

Fig. 14A shows the square wave voltammograms recorded for different concentration of ascorbic acid 0.1 to 0.95 mmol L^{-1} at a fixed concentration of APAP (10 µmol L^{-1}). **Fig. 14B** shows a regression equation in concentration range of ascorbic acid from 0.1 to 0.95 mmol L^{-1}: I_{pa} (µA) = -1.54603 + 18.66215C (mmol L^{-1}), R^2 = 0.98398 and a linear characteristics was observed in concentration range of AA from 0.4 – 0.95 mmol L^{-1}, suggesting that activation of GCE effectively catalyze the determination of AA in the presence of APAP.

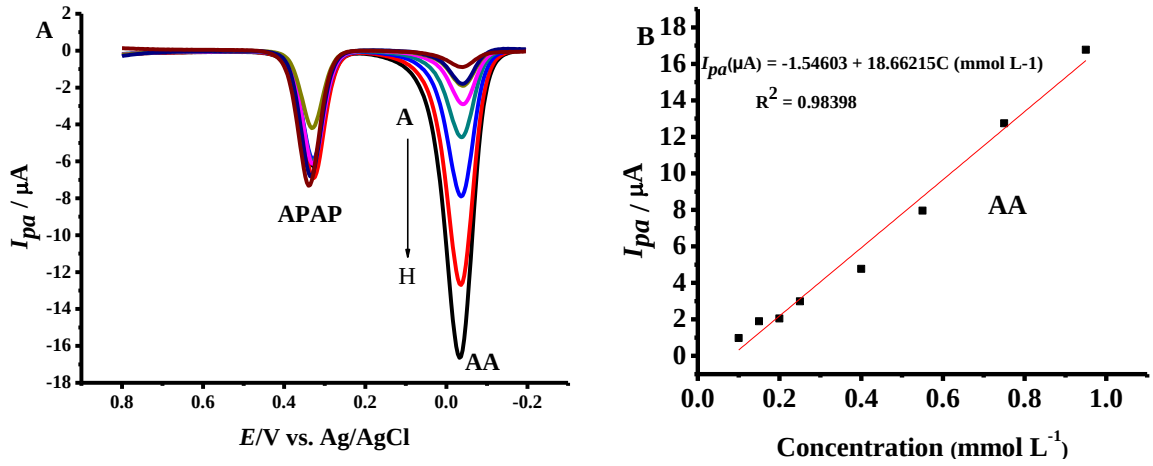


Figure 15. (A) Square wave voltammograms of AA at AGCE in the presence of 10 µmol L^{-1} APAP in 0.1 mol L^{-1} PBS at pH 7.0, AA concentrations (from 1 to 8): 0.1, 0.15, 0.2, 0.25, 0.4, 0.55, 0.75, and 0.95 mmol L^{-1}. (B) Calibration plot of oxidative peak current I_{pa} (µA) *versus* concentration of AA (mmol L^{-1}), other conditions as **Fig. 12**.

The detection limit was calculated using the formula $3\delta/M$, where δ is the standard deviation of the blank under the same conditions as for the standard sample analysis which was taken from eight repeats (n = 8) and M is the slope obtained from the calibration plot. The detection limit of APAP was 0.155 µmol L^{-1}, standard deviation (0.02642 µA) and slope from the calibration plot was (0.51121 µA / µmol L^{-1}). Similarly, the detection limit of AA was 1.89 µmol L^{-1}, standard deviation (0.0118 µA) and slope (18.66215 µA / mmol L^{-1}). The limit of quantification was also calculated by the relationship $10\delta/M$. The quantification limit of APAP was 0.517 µmol L^{-1} and 6.32 µmol L^{-1} for AA. The *LOD* and *LOQ* values showed that activation has highest catalytic ability in comparisons with the previous methods **(Table. 3)** for the determination of both paracetamol and ascorbic acid without chemical modification.

4. Recovery study

The square wave voltammetry method was applied to cheek the validity of the proposed method by determining the concentration of APAP and AA present in a commercial drug formulation paracetamol + vitamin C SANDOZ (500 mg APAP / 300 mg AA). The samples obtained from the dissolution of paracetamol + vitamin C SANDOZ were diluted to the standard paracetamol and

ascorbic acid these in the linear range of the concentrations of the standard samples in the calibration curve. The concentrations of APAP and AA in drug formulation (paracetamol + vitamin C SANDOZ) were determined from the calibration curves plotted as standard concentration added versus peak current. The results showed that the recovery tests of APAP and AA were 101.05% and 95%, respectively, indicating that the method developed in this work had excellent sensitivity and selectivity for detecting APAP and AA in commercial drug formulation. In addition, the recovery studies show that the drug excipients do not significantly interfere with the proposed method. The amount of paracetamol and ascorbic acid on the pharmaceutical formulations were 484.95 mg APAP / 299.95 mg AA, indicating in a good agreement with the amount of paracetamol + vitamin C SANDOZ provided by the manufacturers (500 mg APAP / 300 mg AA).

5. Interference study

In order to evaluate the selectivity of the method the electrochemical behavior of the coexisting electroactive species, which often cause serious interference with the determination of APAP (0.1 mmol L^{-1}), such as AA (0.5 mmol L^{-1}) and uric acid (0.1 mmol L^{-1}) were investigated by using square wave voltammetry. As shown, **(Fig. 15a)** the oxidation potential of ascorbic acid (E_{pa} = -0.02 V) at the AGCE was found to be more negative than that of APAP (E_{pa} = 0.33 V), suggesting that it is possible to quantify or the selected method had good selectivity towards determination of APAP and AA. Therefore, it could be possible for selective detection of APAP in the presence of AA from the selected method. Similarly, for the simultaneous determination of APAP and AA **(Fig. 15b)** a uric acid (0.1 mmol L^{-1}) was added to a mixture containing (0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA). The oxidation peak currents were measured however, the addition of equal concentration of uric acid (UA) to APAP (0.1 mmol L^{-1}) lowered the peak current of APAP and broadness the peak shape with shoulder **(Fig. 15b)**.

Peak to peak separation of paracetamol and uric acid were small as a result, determination of APAP in the presence of equal concentration of UA is not possible because their redox potentials are extremely close due to uric acid oxidized near the oxidation potential of APAP using AGCE. In addition, the corresponding oxidation peak currents obtained in the presence of uric acid were compared with the oxidation peak currents in the absence of uric acid. The percent change on the

peak response of APAP was 21.2% and less than 5% for AA (1.7%). Hence, uric acid interfered the determination of APAP.

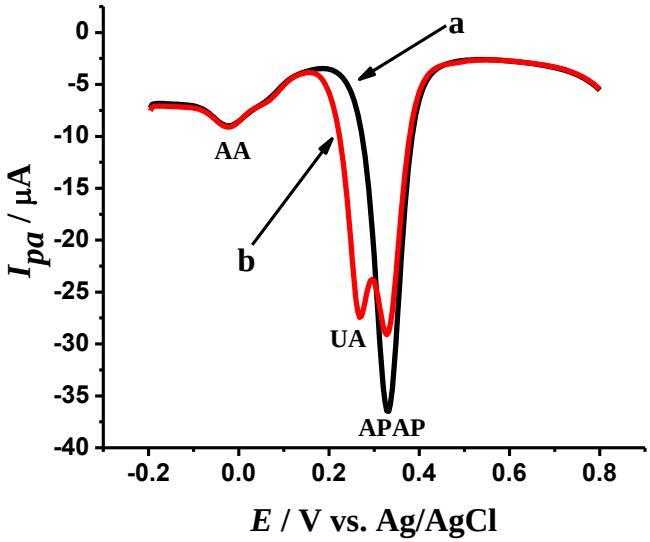


Figure 16. Square wave voltammograms of (a) mixture of 0.1 mmol L^{-1} APAP and 0.5 mmol L^{-1} AA, (b) mixture of 0.1 mmol L^{-} APAP, 0.1 mmol L^{-1} UA and 0.5 mmol L^{-1} AA using AGCE.

6. Comparison with other electrochemical methods

A comparison between the analytical performance of the proposed method for determination of paracetamol and ascorbic acid are given in **Table 3**. As shown from **Table 3** most the selected methods conducted using modified electrodes. The preparation of modified electrodes is time consuming because it involves varies steps in incorporation of the different modifier to the electrode surface.

However, unlike the chemically modified methods the activation method was cost effective, rapid, sensitive, lower risk of measurement errors and operational skills of analyst. In addition the good linear concentration ranges and low detection limits were calculated without any chemical modification. This shows that the selected method had excellent catalytic ability towards the determination of APAP and AA.

Table 3. Comparison of simultaneous determination of APAP and AA with other methods

Modified electrode	Method	Linear range (µM)	LOD (µM)	pH	References
MWCNT/CPE	**DPV**	**APAP; 2.0 to 400**	**0.80**	**PBS (6)**	**[32]**
		AA; 0.02 to 140	**0.009**		
Al/Pd	**DPV**	**APAP; 100 to 5000**	**5.00**	**ABS (6)**	**[33]**
		AA; 100 to 300	**5.00**		
SWCNT/CCE	**DPV**	**APAP; 0.2 to 150**	**0.12**	**PBS (7)**	**[34]**
		AA; 5.0 to 700.0	**3.00**		
Au/MWCNT/GCE	**DPV**	**APAP; 0.09 to 35**	**0.03**	**BRBS (6.0)**	**[35]**
		AA; 1.0 to 150	**0.76**		
(GCEMWCNT-Polyhistidine)	**DPV**	**APAP; 0.25 to 5.0**	**0.032**	**PBS (7.4)**	**[36]**
		AA; 25 µM to 1.50	**0.76**		
(Thionine – (MWCNT/CPE)	**DPV**	**APAP; 0.1to 100**	**0.05**	**ABS (4)**	**[37]**
		AA; 1.0 to 100	**0.30**		
BDDE	**DPV**	**APAP; 10 to 100**	**0.85**	**BRBS (1.6)**	**[38]**
		AA; 10 to 100	**0.77**		
(Redox polimer-MWCNT)	**Amperometry**	**APAP: 0.25 to 1.50**	**1.00**	**PBS (7.4)**	**[64]**
		AA: 0.1 to 100	**2.01**		
AGCE	**SWV**	**APAP: 10 to100**	**0.155**	**PBS (7)**	**This work**
		AA: 400 to 950	**1.890**		

3. CONCLUSIONS

Activated glassy carbon electrode has been used successfully for the electrocatalytic oxidation and determination of APAP and AA at a physiological pH phosphate buffer solution. It was found that the oxidation and reduction peak current of both APAP and AA were improved significantly and the oxidation peak shifted towards less positive potentials at the surface of the AGCE compared to the untreated GCE which indicating that AGCE displays excellent electro-catalytic prop erty towards the determination of APAP and AA. In addition, the AGCE facilitates the determination of APAP and AA with good sensitivity electivity and enhancement of peak currents compared to the bare GCE. The effect of scan rate studies indicates that the catalytic reaction was controlled surface adsorption process. The calibration plots of anodic peak current *versus* the concentration of APAP and AA a linear behavior in the range of 10 to 100 µmol L^{-1} and 0.4 to 0.95 mmol L^{-1}, respectively, observed. The practical analytical utility of the method was successfully demonstrated in the analysis of pharmaceutical formulations: paracetamol + Vitamin C SANDOZ with a recovery of APAP 101.05 % and AA 95 %. The interfering study showed that uric acid interfere paracetamol. The most simple, selective, cost effective, fast and sensitive electrochemical method for the determination of APAP and AA, interesting for the further application such as quality control of medicines in pharmaceutical and related industries.

4. REFERENCES

[1]. Siddiqui, R.M., A.Z., & Rahman, N. (2013). Analytical techniques in pharmaceutical analysis. *Arabian Journal of Chemistry*, 10, 1409-1421.

[2]. Kang, X., Wang, J., Wu, H., Liu, J., Aksay, A. I., & Lin, Y. (2010). A grapheme based electrochemical sensor for sensitive detection of paracetamol. *Talanta*, 81, 754-759.

[3]. Jóźwiak, M.B., & Nowak, Z.J. (2013). Paracetamol mechanism of action, applications and safety concern. *Acta Poloniae Pharmaceutica*, 71, 11-23.

[4]. Gete, G.T., & Kassaw, M.M. (2016). Cyclic voltammetric study of paracetamol at nickel hexacyanoferrate modified carbon paste electrode. *Journal of Natural Sciences Research*, 6, 2224-3145.

[5]. Babaei, A., Dehdashti, A., & Afrasiabi, M. (2011). Development of a method for a sensitive simultaneous determination of dopamine and paracetamol in biological samples and pharmaceutical preparations. *International Journal of Electrochemistry*, 2011, 1-6.

[6]. Tsvetkova, B., Pencheva, I., Zlatkov, A., & Peikov, P. (2012). Simultaneous high performance liquid chromatography determination of paracetamol and ascorbic acid in tablet dosage forms. *African Journal of Pharmacy and Pharmacology*, 6, 1332-1336.

[7]. Suryan, L.A., Bhusari, K.V., Rasal, S.K., & Dhaneshwar, R.S. (2011). Simultaneous quantitation and validation of paracetamol, phenylpropanolamine hydrochloride and cetirizine hydrochloride by RPHPLC in bulk drug and formulation. *International Journal of Pharmaceutical Science and Drug Research*, 3, 303-308.

[8]. Adeneye, A.A., & Olagunju, O.J. (2008). Protective effect of oral ascorbic acid (Vitamin C) against Acetaminophen-induced hepatic injury in rats. *African Journal of Biomedical Research*, 11, 183-190.

[9]. Shahrokhian, S., & Saberi, S.R. (2011). Voltammetric determination of acetaminophen in the presence of codeine and ascorbic acid at layer bylayer MWCNT/hydroquinone sulfonic acid-overoxidized polypyrrole modified glassy carbon electrode. *International Journal of Electrochemistry*, 2011, 1-10.

[10]. Atta, F.N., Galal, A., & Azab, M.S. (2011). Electrochemical determination of paracetamol using gold nanoparticles application in tablets and human fluids. *International Journal of Electrochemical Science*, 6, 5082-5096.

[11]. Sadok, I., & Tyszczuk, K.R. (2015). New, simple and sensitive voltammetric procedure for determination of paracetamol in pharmaceutical formulations. *Insights Anal Electrochem*, 1, 1.

[12]. Goyal, N.R., & Singh, P.S. (2006). Voltammetric determination of paracetamol at C 60-modified glassy carbon electrode. *Electrochemical Acta*, 51, 3008-3012.

[13]. Yin, H., Shang, K., Meng, X., & Ai, S. (2011). Voltammetric sensing of paracetamol, dopamine and 4-aminophenol at a glassy carbon electrode coated with gold nanoparticles and an organophillic layered double hydroxide. *Microchimica Acta*, 175, 39-46.

[14]. Norouzi, P., Dousty, F., Ganjali, R.M., & Daneshgar, P. (2009). Dysprosium nanowire modified carbon paste electrode for the simultaneous determination of naproxen and paracetamol. *International Journal Electrochemical Science*, 4, 1373-1386.

[15]. Li, C., Zhan, G., Yang, Q., & Lu, J. (2006). Electrochemical investigation of acetaminophen with a carbon nano-tube composite film electrode. *Bulletin of Korean Chemical Society*, 27, 1854-1860.

[16]. Adikwu, E., & Deo, O. (2013). Hepatoprotective effect of vitamin C (ascorbic acid). *Pharmacology and Pharmacy*, 4, 84.

[17]. Golonka, I., Kawacki, A., & Musial, W. (2015). Stability studies of a mixture of paracetamol and ascorbic acid, prepared extempore, at elevated temperature and humidity conditions. *Tropical Journal of Pharmaceutical Research*, 14, 1315-1321.

[18]. Goh, K. J., Tan, T.W., Lim, T.F., & Maamor, M.A.N. (2008). Electrochemical oxidation of ascorbic acid mediated by carbon nanotubes/Li+/carbon paste modified solid electrode. *Malaysian Journal of Analytical Sciences*, 12, 480-485.

[19]. Beitollahit, H., Karimi, H.M., & Sheikhshoaie, I. (2012). Simultaneous voltammetric determination of ascorbic acid and uric acid using a modified multiwalled carbon nanotube paste electrode. *Caspian Journal of Chemistry*, 1, 17-29.

[20]. Matei, N., Birghila, S., Popescu, V., Dobrinas, S., Soceanu, A., Oprea, C., & Magearu, V. (2008). Kinetic study of vitamin C degradation from pharmaceutical products. *Romina Journal of Physics*, 53, 343-351.

[21]. Gioia, M.G., Andreatta, P., Boschetti, S., & Gatti, R. (2008). Development and validation of aliquid chromatographic method for the determination of ascorbic acid,dehydroascorbic acid, and acetaminophen in pharmaceuticals. *J. Pharm.Biomed. Anal*, 48, 331-339.

[22]. Akay, C., Gumusel, B., Degim, T., Tartilmis, S., & Cevheroglu, S. (1999). Simultaneous determination of acetaminophen, acetylsalicylic acid, and ascorbic acid in tablet form using HPLC, Drug Metabol. *Drug Interact*, 15, 197-205.

[23].Thomis, R., Roets, E., & Hoogmartens, J. (1984). Analysis of tablets containing aspirin,acetaminophen, and ascorbic acid by high-performance liquid chromatography. *J. Pharm. Sci*, 73, 1830-1833.

[24]. Sarakbi, A., Aydogmus, Z., Sidali, T., Gokce, G., & Kauffmann, J.M. (2011). Simultaneous determination of paracetamol and ascorbic acid in pharmaceutical formulations by LC

coupled to a screen printed carbon based amperometric detector. *Electroanalysis*, 23, 29-36.

[25].Wang, J., Chatrathi, P.M., Tian, B., & Polsky, R. (2000). Microfabricated electrophoresis chips for simultaneous bioassays of glucose, uric acid, ascorbic acid, and acetaminophen. *Anal. Chem.* 72, 2514-2518.

[26].Sandulescu, R., Mirel, S., & Oprean, R. (2000). The development of spectrophotometric and electroanalytical methods for ascorbic acid and acetaminophen and their applications in the analysis of effervescent dosage forms. *J. Pharm. Biomed. Anal.* 23, 77-87.

[27]. Skrovánkova, S., Mlcek, J., Sochor, J., Baron, M., Kynicky, J., & Jurikova, T. (2015). Determination of ascorbic acid by electrochemical techniques and other method. *International Journal of Electrochemical Science, 10, 2421-2431.*

[28]. Suresh, E., Sundaram, K., Kavitha, B., & Kumar, S.N. (2016). Square wave voltammetry sensing of ibuprofen on glassy carbon electrode. *International Journal of Pharmaceutical Technology Research,* 9, 182-188.

[29]. Settle, F.A. (1997). Handbook of instrumental techniques for analytical chemistry. *Prentice Hall PTR, UK,* 711-725.

[30]. Zanello, P. (2003). *Inorganic electrochemistry: theory, practice and applications*. *Royal Society of Chemistry*, Cambridge, *UK*, 49-134.

[31].Monk, P.M. (2008). Fundamentals of electroanalytical chemistry. *Manchester Metropolitan University, Manchester, UK,* 156-182.

[32]. Keyvanfard, M., Shakeri, R., Karimi, H.M., & Alizad, K. (2013). Highly selective and sensitive voltammetric sensor based on modified multiwall carbon nanotube paste electrode for simultaneous determination of ascorbic acid, acetaminophen and tryptophan. *Materials Science and Engineering*, 33, 811-816.

[33]. Pournaghi-Azar, H.M., & Saadatirad, A. (2010). Simultaneous determination of paracetamol, ascorbic acid and codeine by differential pulse voltammetry on the aluminum electrode modified by thin layer of palladium. *Electroanalysis,* 22, 1592-1598.

[34]. Habibi, B., Jahanbakhshi, M., & Pournaghi, H.M.A. (2011). Differential pulse voltammetric simultaneous determination of acetaminophen and ascorbic acid using single-walledcarbon nanotube modified carbon ceramic electrode. *Analytical Biochemistry*, 411, 167-175.

[35]. Madrakian, T., Haghshenas, E., & Afkhami, A. (2014). Simultaneous determination of tyrosine, acetaminophen and ascorbic acid using gold nanoparticles/multiwalled carbon nanotube/glassy carbon electrode by differential pulse voltammetric method. *Sensors and Actuators*, 193, 451-460.

[36]. Dalmasso, R.P., Pedano, L.M., & Rivas, A.G. (2012). Electrochemical determination of ascorbic acid and paracetamol in pharmaceutical formulations using a glassy carbon electrode modified with multi-wall carbon nanotubes dispersed in polyhistidine. *Sensors and Actuators*, 173, 732-736.

[37]. Shahrokhian, S., & Asadian, E. (2010). Simultaneous voltammetric determination of ascorbic acid, acetaminophen and isoniazid using thionine immobilized multi-walled carbon nanotube modified carbon paste electrode. *Electrochimical Acta, 55*, 666-672.

[38]. Radovan, C., Cofan., C.,& Cinghita, D.(2008).Simultaneous determination of acetaminophen and ascorbic acid at an unmodified boron-doped diamond electrode by differential pulse voltammetry in buffered media. *Electroanalysis*, 20, 1346-1353.

[39]. Ensafi, A.A., Karimi, H.M., & Mallakpour, S. (2012). Simultaneous determination of ascorbic acid, acetaminophen, and tryptophan by square wave voltammetry using N- (3, 4-Dihydroxyphenethyl) -3, 5-Dinitrobenzamide-modified carbon nanotubes paste electrode. *Electroanalysis*, 24, 666-675.

[40]. Miner, J.D., Rice, R.J., Riggin, M.R., & Kissinger, T.P. (1981). Voltammetry of acetaminophen and its metabolites. *Analitical Chemistry*, 53, 2258-2263.

[41]. Yilmaz, S., Sadikoglu, M., Saglikoglu, G., Yagmur, S., & Askin, G. (2008). Determination of ascorbic acid in tablet dosage forms and some fruit juices by DPV. *International Journal of Electrochemical Science*, 3, 1534-1542.

[42]. Banan, D., Tan, T.W., Sulaiman, Y., Yusri, F.M., Zidan, M., & Ghani, A.S. (2013). Electrochemical oxidation of ascorbic acid using MgB 2-MWCNT modified glassy carbon electrode. *International Journal of Electrochemical Science*, 8, 12519-12530.

[43]. Gete, G.T., and Kassaw, M.M. Determination of paracetamol and kinetic parameters using nickel hexacyanoferrate modified carbon paste electrode. *International Journal of Advanced Research*, 4, 1087-1093.

[44]. Tripathi, P.R., Singh, B., Bisht, S.S., & Pandey, J. (2009). L-Ascorbic acid in organic synthesis. *Current Organic Chemistry*, 13, 99-122.

[45]. Barrita, J.L.S., & Sánchez, S.S.D. M (2013). Antioxidant role of ascorbic acid and its protective effects on chronic diseases. *Rejeka Intech*, 449-481.

[46]. Abdel, G.M.M., Ghoneim, M.M., El, D.S.H., Hattori, T., & Matsuda, A. (2014). Anodic stripping voltammetry determination of lead ions using highly sensitive modified electrodes based on multi-walled carbon nanotube. *Journal of Chemistry*, 2, 25-43.

[47]. Gomathi, H., Chandra, N., & Sharma, R.L. (1991). Pretreatment of glassy carbon electrode leading to surface modification in ferro/ferric cyanide in pottasium chloride medium. *Bulletin of Electrochemistry*, 7, 477-479.

[48]. Weisshaar, H.D., & Kuwana, T. (1985). Considerations for polishing glassy carbon to a scratch-free finish. *Analytical Chemistry*. 57, 2759-2763.

[49]. Cavalheiro, G.T.É., Brett, A.M.C., Oliveira-Brett, M.A., & Fatibello, F.O. (2012). Bioelectroanalysis of pharmaceutical compounds. *Bioanalytical Reviews*, 4, 31-53.

[50]. Carvalho, C.R., Gouveia, C.C., & Brett, A.M.C. (2010). Glassy carbon electrodes modified by multiwalled carbon nanotubes and poly (neutral red). *Analytical and Bioanalytical chemistry*, 398, 1675-1685.

[51]. Xu, Q., Guo, X.R., Wang, Y.C., & Hu, Y.X. (2007). Application of activated glassy carbon electrode for the detection of nuciferine in lotus leaves. *Talanta*, 73, 262-268.

[52]. Dekanski, A., Stevanovic, J., Stevanovic, R., Nikolic, Z.B., & Jovanovic, M.V. (2001). Characterization and electrochemical activation of glassy carbon electrodes. *Carbon*. 39, 1195-1205.

[53]. Ricci, F., Palleschi, G., Yigzaw,Y., Gorton,L., Ruzgas, T., & Karyakin, A. (2003). Investigation of the effect of different glassy carbon materials on the performance of Prussian blue badsed sensors for hydrogen peroxide. *Journal of Electroanalyis*. 3, 15.

[54]. Farghaly, A.O., Hameed, A.S., & Nawwas, A.H. (2014). Analytical application using modern electrochemical techniques. *International Journal of Electrochemical Science*. 9, 3287-3318.

[55]. Sanghavi, J.B., & Srivastava, K.A. (2011). Simultaneous voltammetric determination of acetaminophen and tramadol using Dowex50wx2 and gold nanoparticles modified glassy carbon paste electrode. *Analytica Chimica acta*, 706, 246-254.

[56]. Protti, P. (2001). Introduction to modern voltammetric and polarographic analisys techniques. *Amel Electrochemistry, 4th edition., New York, USA*, 9-18.

[57]. Chethana, K.B., Basavanna, S., & Naik, A.Y. (2012). Voltammetric determination of diclofenac sodium using tyrosine-modified carbon paste electrode. *Industrial and Engineering Chemistry Research*, 51, 10287-10295.

[58]. Zayed, M.I.S., & Arida, M.A.H. (2013). Preparation of carbon paste electrodes and its using in voltammetric determination of amiloride hydrochloride using in the treatment of high blood pressure. *International Journal of Electrochemical Science*, 8, 1340-1348.

[59]. Filik, H., Çetintaş, G., Avan, A.A., Koç, N.S., & Boz, İ. (2013). Electrochemical sensing of acetaminophen on electrochemically reduced graphene oxide-nafion composite film modified electrode. *International Journal of Electrochemical Science*, 8, 5724-5737.

[60]. Ciltas, U., Yilmaz, B., Kaban, S, Akcay, K.B., & Nazik, G. (2015). Square wave Voltammetric determination of diclofenac in pharmaceutical preparations and human serum. *Iranian Journal of Pharmaceutical Research*, 14, 715-722.

[61]. Filik, H., Avan, A. A., Aydar, S., & Çetintaş, G. (2014). Determination of acetaminophen in the presence of ascorbic acid using a glassy carbon electrode modified with poly (caffeic acid). *International Journal Electrochemical Society*, 9, 148-160.

[62]. Ren, W., Luo, H. Q., & Li, N. B. (2006). Simultaneous voltammetric measurement of ascorbic acid, epinephrine and uric acid at a glassy carbon electrode modified with caffeic acid. *Biosensors and Bioelectronics*, 21, 1086-1092.

[63]. Sheng, Z. H., Zheng, X. Q., Xu, J. Y., Bao, W. J., Wang, F. B., & Xia, X. H. (2012). Electrochemical sensor based on nitrogen doped graphene: simultaneous determination of ascorbic acid, dopamine and uric acid. *Biosensors and Bioelectronics*, *34*, 125-131.

[64]. Havens, N., Trihn, P., Kim, D., Luna, M., Wanekaya, A. K., & Mugweru, A. (2010). Redox polymer covalently modified multiwalled carbon nanotube based sensors for sensitive acetaminophen and ascorbic acid detection. *Electrochimica acta*, 55, 2186-2190.